Added Value of an Oropharyngeal Swab in Detection of Viruses in Children Hospitalized with Lower Respiratory Tract Infection[▽]

Laura L. Hammitt,[1,2]* Sidi Kazungu,[1] Steve Welch,[3] Anne Bett,[1] Clayton O. Onyango,[1] Rory N. Gunson,[4] J. Anthony G. Scott,[1,2] and D. James Nokes[1,5]

KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya[1]; Nuffield Department of Clinical Medicine, University of Oxford, Oxford, United Kingdom[2]; Health Protection Agency, London, United Kingdom[3]; West of Scotland Specialist Virology Centre, Gartnavel General Hospital, Glasgow, Scotland[4]; and School of Life Sciences, University of Warwick, Coventry, United Kingdom[5]

Paired nasopharyngeal and oropharyngeal swabs collected from 533 children hospitalized with lower respiratory tract infection were assessed by multiplex reverse transcription-PCR. Oropharyngeal swabs increased the number of viral infections detected by 15%, compared to collection of a nasopharyngeal swab alone. This advantage was most pronounced for detection of influenza, parainfluenza, and adenovirus.

Samples from the nasopharynx (NP) or oropharynx (OP) are often used to diagnose respiratory tract infections. Using modern molecular diagnostics, several studies have described the performance of an NP flocked swab sample in detecting respiratory viruses (1, 3, 10) (P. Munywoki et al., submitted for publication). OP swab samples have been found to be consistently less sensitive than NP samples for a variety of viruses; however, maximum sensitivity is attained by using multiple types of samples (6, 7, 8). Through a standardized multisite study, we aim to describe the etiology of respiratory tract infection in children in developing countries. Before beginning this study, we evaluated the added diagnostic value of an OP swab over and above diagnoses made by an NP swab.

Children aged 1 day to 12 years admitted with a lower respiratory tract infection (LRTI) to Kilifi District Hospital, Kenya, between October 2009 and August 2010 were eligible for participation. LRTI was defined as a history of cough and/or difficulty breathing and at least one accompanying respiratory sign (i.e., indrawing, nasal flaring, central cyanosis, head nodding, or tachypnea). Written informed consent was obtained from the parent or guardian of each participant. Paired NP and OP samples were collected from each child on the day of admission. An NP flocked swab (Copan Diagnostics, Inc., Italy) was passed into the posterior nasopharynx via the nostril, rotated 2 to 3 times, withdrawn, and placed in a vial of transport medium (25 mg/ml veal infusion, 5 mg/ml bovine factor V, 50 mg/ml gentamicin, and 250 μg/ml amphotericin B; prepared in-house). A polyurethane foam-tipped OP swab (Sigma-swab; Medical Wire & Equipment, England) was inserted into the mouth, swabbed over the tonsils and posterior pharynx, withdrawn, and placed in a vial of transport medium (as described above). Samples were stored at 4°C until analyzed. All samples were analyzed within 48 h of collection.

Nucleic acid was extracted from samples using either a Qiagen viral RNA miniprep kit (Qiagen, United Kingdom; sample volume = 140 μl) or a total nucleic acid extraction kit (Roche Applied Science, Germany) with a MagNA Pure LC32 automated NA extractor (sample volume = 200 μl). Paired samples were extracted using the same method. To ensure coextraction of both DNA and RNA, no DNase step was included. To allow for extraction of nucleic acid from any intracellular pathogens, such as *Mycoplasma pneumoniae*, the samples were not spun down to pellet cellular debris prior to extraction.

Extracted samples were tested by a modified multiplex (MPX) reverse transcription (RT)-PCR assay in triplexes for 16 respiratory pathogens: respiratory syncytial viruses (RSV) A and B, adenovirus, rhinovirus, human metapneumovirus (HMPV), coronaviruses (NL63, OC43, 229E), parainfluenza viruses (PIV) 1 to 4, influenza viruses (A, B, C), and *Mycoplasma pneumoniae* (4). The complete list of primer and probe sequences is shown in Table 1. The primers were obtained from either Sigma-Genosys (Sigma Aldrich, United Kingdom) or MWG (Eurofins MWG Operon, United Kingdom). The hydrolysis probes using black hole quencher (BHQ) molecules were obtained from MWG (Eurofins MWG Operon, United Kingdom), and the hydrolysis probes using 6-carboxytetramethylrhodamine (TAMRA) or minor-groove binder nonfluorescent quencher (MGBNFQ) molecules were obtained from ABI (Applied Biosystems, United Kingdom). Reverse transcription was carried our for 20 min at 50°C. Platinum *Taq* polymerase was activated at 95°C for 5 min, followed by 40 cycles of PCR performed at 95°C for 15 s and 60°C for 30 s. The assays were run using the Qiagen Quantifast multiplex RT-PCR kit (Qiagen, United Kingdom) in triplex sets and analyzed on an ABI 7500 system (Applied Biosystems, United Kingdom) using SDS 3.2 software. The control materials were either RNA supplied by the West of Scotland Specialist Virology Centre (Gartnavel Hospital, Glasgow, United Kingdom) or PCR products generated in-house. The positive controls consisted of viral RNA or PCR products for influenza viruses A, B, and C; parainfluenza viruses 1 to 4; human coronaviruses

---

* Corresponding author. Mailing address: KEMRI Wellcome Trust Research Programme, P.O. Box 230, Kilifi, Kenya 80108. Phone: 254 41 7522063. Fax: 254 41 7522390. E-mail: llhammitt@gmail.com.

TABLE 1. Primer and probe sequences in the multiplex real-time RT-PCR respiratory screen[a]

Target	Target protein	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	Probe sequence (5'-3')	Product size (bp)
Influenza virus A	Matrix	AAGACAAGACCAATYCTGTCACCTCT	TCTACGYTGCAGTCCYCGCT	FAM-TYACGGCTCACCGTGCCCAGTG-BHQ1	93
Influenza virus A	NS	ATGATCTTACAGTGGAGGATGAAGAA	CGAATTGGCTTGRATGTCCTT	CY5-ATGGCCATCGGATCCTCAAYTCACTCT-BHQ2	91
Influenza virus B	Matrix	GGCAAGGGACATGCTGAAYA	TCCAGCTGCYTTCATTTGCTTT	VIC-CTCTTCCTTCGTGATTTTTTCAAA-MGBNFQ	85
Influenza virus C	Matrix	GCYGTYAGCTTCAGTCAATTCAA	TCCAGCATTGTCTGAAAATTGC	VIC-CAACATTTAGAAAACCTTCT-MGBNFQ	69
HMPV A	Fusion	GCYGTYAGCTTCAGTCAATTCAA	GTTATCCCTGCATTGTCTGAAAACT	VIC-CGCACAAACATTTAGGAAATCTTCT-MGBNFQ	74
HMPV B	Fusion (Common with A)	GTGATTTAAACCGGTAATTTCTCA	CCTTGTTCCTGCAGGCTATTACAGA	FAM-ACCTATGACATCAACGAC-MGBNFQ	83
PIV 1	HN	ATGAAAACCATTTACCTAAGTGATGGA	CCTCCYGGTATRGCAGTGACTGAAC	VIC-TCAATCGCAAAAGC-MGBNFQ	68
PIV 2	HN	CAGGGGATATAYATYAAYAAAGGCAAAA	CGGGRCACCCCAGTTGTG	FAM-TGGRTGTTCAAGACCTCATAYCCGGAGAAA-BHQ1	101
PIV 3	HN	CAGAYAACATCAATCAGTGGGTTACAAA	TGTAGGTYATGAGTCGCCCAAARA	CY5-CCMATCACAAGGCYCAGAAATYCAAAGTCGT-BHQ2	80
PIV 4	Fusion	CAGTCAAATGGGCTYATTCGATGCA	AAAGGGCTATAAAGAAGAAATAAGGTATTCT	FAM-CCCTGACGACCACGTTGTGTGTTCA-BHQ1	76
HCV 229E	Nucleocapsid	CGATGAGGGCTATTCCGACTAGGT	CCTTCCTGAGCGCCTTCAATATAGTAAGC	CY5-TCCGCCTGGCACCGGTACTCCCT-BHQ3	76
HCV OC43	Nucleocapsid	ACGACTTCTATTTGAAGCATGATGATATTAA	AGCAGTCTAAATGTTTATACTTTAAAAACTAAC	VIC-ATTGCCAAGGTGCTCCTAAAAGTACACAGGTGTT-TAMRA	103
HCV NL63	1a	AGATCAACATTCTCTGTCATCCAGCAA	TTCTGCACATCATTAATTAGGAG	FAM-CACCATCCAAACGGGAGCAAGGAGAGAT-BHQ1	84
RSV A	NP	AAGATGCAAAATCATAAAATTCACAGGA	TGTATCCAGCATCTTTTAAGTA	VIC-TTTCCCTTCCTAAACCTGGACATA-TAMRA	103
RSV B	NP	AAGATGCAAAATCATAAAATTCACAGGA	CAAAGTTAGTCGGCCTCCATCC	FAM-CACCATCCAACGGAGC-BHQ1	103
Rhinovirus	5'-UTR	TGGACAGGGGTTGTGAAGAGC	CAAAGTAGTCGGTCCCATCC	VIC-TCCTCCGGCCCCTGAATG-TAMRA	144
Adenovirus	Matrix	GCCACSGTGGGGTTTCTAAACTT	GCCCCAGTGGTCYTACATGCACATC	CY5-TGCACCAGACCCGGGCTACTCCGA-BHQ2	132
Mycoplasma pneumoniae	Cytadhesin P1	AAGCAGGGAGTGAGCGGAAACAC	CACCCACCATCATTCCCGTATT	CY5-CTCCACCAACAACCTGCGCGGTA-BHQ2	73

[a] BHQ, black hole quencher; NS, nonstructural; HN, hemagglutinin; NP, nucleoprotein; UTR, untranslated region; HMPV, human metapneumovirus; PIV, parainfluenza virus; HCV, human coronavirus; RSV, respiratory syncytial virus; FAM, 6-carboxyfluorescein.

229E, OC43, and NL63; HRV; human metapneumoviruses A and B; and RSV A and B. The control material for adenovirus and *Mycoplasma pneumoniae* consisted of plasmids cloned with PCR target sequences. Specimens were deemed to be positive for a particular pathogen if the cycle threshold value was less than 35. Samples that were positive for influenza virus A by MPX PCR were tested for 2009 H1N1 influenza virus with a real-time RT-PCR assay (CDC) (2).

To estimate sensitivity, the gold standard for the presence of a pathogen was defined as a positive result in either the NP or the OP swab. All 95% confidence intervals (CIs) were estimated using exact methods. The added value of an OP swab was calculated as the ratio (number of infections detected by either swab/number of infections detected by an NP swab) minus one and expressed as a percentage. Statistical analyses were performed using STATA 11.0 (Stata Corp., College Station, TX).

Paired NP and OP swabs were collected from 533 children admitted to the hospital with signs and symptoms of lower respiratory tract infection. The median age (interquartile range) of participants was 18 months (4 to 21 months), and 297 (56%) were boys. A virus was detected in 339 (64%) NP samples and 268 (50%) OP samples. Overall, the OP sample increased the number of children detected to have a viral infection by 8.8% (Table 2) (8% [22 children] in those aged <24 months, 10% [5 children] in those aged 24 to 59 months, and 18% [3 children] in those aged ≥60 months). Collection of an OP sample increased the total number of different viruses detected by 15.3% (Table 2) (13% [45 viruses] in those aged <24 months, 27% [15 viruses] in those aged 24 to 59 months, 24% [4 viruses] in those aged ≥60 months). Coinfection with two or more viruses was detected in 66 (12%) NP samples and 46 (9%) OP samples. The viruses most commonly detected in children with coinfection were rhinovirus, parainfluenza virus, and adenovirus. The added value of the OP sample varied by virus from 9% (rhinovirus) to 31% (adenovirus). None of the samples tested positive for *Mycoplasma pneumoniae*.

The primary aim of the study was to assess the added diagnostic yield from an OP swab; therefore, the study was not powered to determine whether sensitivity of detection differed between the two collection methods. Despite this, the 95% CIs around the sensitivity estimates suggest that the NP sample was more sensitive for detection of RSV, parainfluenza viruses, coronaviruses, HMPV, and rhinovirus. The sensitivity of an NP swab for all viruses assessed was 86.7% (95% CI of 83.3, 89.6), and the sensitivity of the OP swab was 66.2% (95% CI of 61.8, 70.4).

Other than supportive care, no specific treatment is available for most of the viruses studied. However, accurate detection of influenza infection can be important for case management. In the setting of pandemic influenza, the practice of sampling both the NP and OP has been recommended, but the value of double sampling has not been clearly established (5, 11). OP swabs were more sensitive than nasal swabs (type of swabs not specified) for detection of the 2005 H5N1 influenza virus (5); however, a study conducted during the 2009 influenza epidemic in Thailand found that Dacron-tipped OP and nasal swabs had comparable sensitivities for detection of H1N1 by RT-PCR (9). In the present study, 6 (18%) influenza virus infections, including 3 (25%) of 12 infections with the 2009

TABLE 2. Viral detection by multiplex PCR of paired NP flocked swabs and OP swabs collected from children aged 1 day to 12 years admitted to Kilifi District Hospital with lower respiratory tract infection ($n = 533$)[b]

Virus	No. of swabs			% increase using OP swab[a] (95% CI)	% sensitivity NP (95% CI)	% sensitivity OP (95% CI)
	NP+ OP+	Only NP+	Only OP+			
RSV A and B	83	24	13	12 (7,20)	89 (82, 94)	80 (72, 87)
PIV 1-4	22	27	10	20 (10, 34)	83 (71, 92)	54 (41, 67)
Influenza A–C	21	6	6	22 (9, 42)	82 (65, 93)	82 (65, 93)
Coronaviruses	12	10	3	14 (3, 35)	88 (69, 98)	60 (39, 79)
Adenovirus	29	22	16	31 (19, 46)	76 (64, 86)	67 (55, 78)
HMPV	34	23	7	12 (5, 24)	89 (79, 96)	64 (51, 76)
Rhinovirus	53	51	9	9 (4, 16)	92 (85, 96)	55 (45, 64)
Any virus detected	238	102	30	8.8 (6.0, 12.4)	91.9 (88.6, 94.5)	72.4 (67.6, 76.9)
All viruses detected	255	163	64	15.3 (12.0, 19.1)	86.7 (83.3, 89.6)	66.2 (61,8, 70.4)

[a] % increase = {[(no. of NP+OP+ swabs) + (no. of only NP+ swabs) + (no. of only OP+ swabs)]/[(no. of NP+OP+ swabs) + (no. of only NP+ swabs)] − 1} × 100.
[b] CI, confidence interval; RSV, respiratory syncytial virus; PIV, parainfluenza virus; HMPV, human metapneumovirus.

H1N1 influenza virus strain, were detected only in the OP sample, suggesting that double sampling is beneficial for maximal detection of this treatable but sometimes fatal illness.

It is not known whether maximal detection of respiratory viral infection is achieved through sampling of multiple anatomical sites or collection of multiple samples from the same site. We elected to sample from different anatomical sites (NP and OP) because of limited data suggesting differential detection of some pathogens (e.g., influenza) from different sites. Additional studies are required to assess whether collection of an additional NP sample, rather than an OP sample, would have resulted in a similar increase in viral detection. The majority of extractions were done using a Qiagen kit, with 7% of samples done by MagNA Pure; reanalysis excluding the latter did not materially alter the findings (results not shown). The types of NP and OP swabs used in this study were selected based on availability and design, with both purporting to offer improved specimen uptake and release. Additional studies are required to assess how swab design and material affect sensitivity.

Selection of a sampling method for detection of respiratory viral infection must balance epidemiological sensitivity against the feasibility, costs, and time required for specimen collection. The collection of an OP swab is relatively quick and simple, and our experience is that it is well accepted; clinical staff, along with most patients and parents, are familiar with the procedure. Reduction in the cost of consumables can be achieved by placing the OP swab and NP swab into the same vial for transport to the lab and testing. For clinical settings or for comprehensive studies of the etiology of lower respiratory tract infection, collection of an OP swab in addition to an NP swab increases the detection of viral infections.

REFERENCES

1. **Abu-Diab, A., et al.** 2008. Comparison between pernasal flocked swabs and nasopharyngeal aspirates for detection of common respiratory viruses in samples from children. J. Clin. Microbiol. **46:**2414–2417.
2. **Centers for Disease Control and Prevention.** October 2009. CDC protocol of real-time RT-PCR for influenza A (H1N1). Centers for Disease Control and Prevention, Atlanta, GA. http://www.who.int/csr/resources/publications/swineflu/realtimeptpcr/en/index.html.
3. **Faden, H.** 2010. Comparison of midturbinate flocked-swab specimens with nasopharyngeal aspirates for detection of respiratory viruses in children by the direct fluorescent antibody technique. J. Clin. Microbiol. **48:**3742–3743.
4. **Gunson, R. N., T. C. Collins, and W. F. Carman.** 2005. Real-time RT-PCR detection of 12 respiratory viral infections in four triplex reactions. J. Clin. Virol. **33:**341–344.
5. **Kandun, I. N., et al.** 2006. Three Indonesian clusters of H5N1 virus infection in 2005. N. Engl. J. Med. **355:**2186–2194.
6. **Lieberman, D., A. Shimoni, A. Keren-Naus, R. Steinberg, and Y. Shemer-Avni.** 2009. Identification of respiratory viruses in adults: nasopharyngeal versus oropharyngeal sampling. J. Clin. Microbiol. **47:**3439–3443.
7. **Lieberman, D., A. Shimoni, A. Keren-Naus, R. Steinberg, and Y. Shemer-Avni.** 2010. Pooled nasopharyngeal and oropharyngeal samples for the identification of respiratory viruses in adults. Eur. J. Clin. Microbiol. Infect. Dis. **29:**733–735.
8. **Robinson, J. L., B. E. Lee, S. Kothapalli, W. R. Craig, and J. D. Fox.** 2008. Use of throat swab or saliva specimens for detection of respiratory viruses in children. Clin. Infect. Dis. **46:**e61–e64.
9. **Suntarattiwong, P., et al.** 2010. Clinical performance of a rapid influenza test and comparison of nasal versus throat swabs to detect 2009 pandemic influenza A (H1N1) infection in Thai children. Pediatr. Infect. Dis. J. **29:**366–367.
10. **Walsh, P., et al.** 2008. Comparison of respiratory virus detection rates for infants and toddlers by use of flocked swabs, saline aspirates, and saline aspirates mixed in universal transport medium for room temperature storage and shipping. J. Clin. Microbiol. **46:**2374–2376.
11. **World Health Organization (WHO).** 2009. Clinical management of human infection with pandemic (H1N1) 2009: revised guidance. World Health Organization, Geneva, Switzerland. http://www.who.int/csr/resources/publications/swineflu/clinical_management_h1n1.pdf.